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Whole body vibration as a safe exercise training method induces no impaired alterations on rat plasma antioxidant biomarkers

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Whole body vibration (WBV) has been regarded as an exercise training method and as a non-pharmacological supportive treatment option appearing to be efficient in chronic disease conditions, such as bone disorders and for cardio-respiratory fitness. Since, data on the safety and efficacy of vibration on oxidative stress parameters are lacking, it was decided to assess the effects of WBV on the plasma antioxidant biomarkers in adult male Wistar rat model. *Methods:* Male Wistar rats weighing 140–180 g, were divided into control and vibration group. Vibration training consisted of vertical sinusoidal whole body vibration for 8 weeks, followed by blood collection. *Results:* The vibrated rats weighed more than the control group (135.0 ± 21.0 vs. 157.0 ± 36.0 g, $P < 0.048$). The plasma Cu and Zn concentrations, vitamin C, uric acid, the activities of antioxidant enzymes, total antioxidant capacity, and malondialdehyde (MDA) levels were similar in the vibration group compared with the controls. The mean of Xantine oxidase level was significantly higher ($P = 0.05$) in the vibration group. No major difference was observed for selected plasma antioxidant parameters. *Discussion:* The potential effects of physiological responses of WBV on several physiological systems are without deteriorations concerning plasma antioxidant status.

Keywords: whole body vibration, male Wistar rat, blood plasma, antioxidant biomarkers

Physical activity as an effective strategy, recommended in general practice, can be used to increase lean mass and bone mass while decreasing fat mass and is also beneficial in improving health status of the individuals (9). Recently, whole body vibration (WBV) has been regarded as an exercise training method with a potential of improving body composition (25). It is suggested that WBV training program as a non-pharmacological supportive treatment option appears to be an efficient alternative treatment for chronic disease conditions, such as bone disorders and cardio-respiratory fitness. High-frequency mechanical strain seems to stimulate bone strength in animals (31, 36). The findings suggest that WBV training might be useful in the prevention of osteoporosis and bone mass loss (15, 16, 28, 29, 34, 35), it improves isometric and dynamic muscle and muscle strength (5, 27, 34) reduces body fat accumulation and serum leptin concentration (20). It is effective in suppressing the atrophy pathway both *in vivo* and *in vitro* and enhances fusion of satellite muscle cells (8), and could be of benefit to patients with multiple sclerosis (30), type 2 diabetic males (2), and may be of

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benefit to arterial function and muscle strength in deconditioned individuals who cannot perform conventional exercise (13).

Recently, the effect of vibration on some plasma biochemical parameters in healthy male Wistar rats was reported (22). It is generally known that conventional exercises such as endurance, ultra endurance exercise, downhill treadmill (muscle-damaging exercise) or a graded exercise treadmill test can increase oxygen utilization and greatly increase the generation of free radicals and enhance damage to muscles and other tissues (14, 18, 23, 33). However, there was no change in the analysis of oxidative stress in mice which were undergone a single session of aerobic exercise (19). It appears that different types of exercises have different impact on the antioxidant system.

Since the effects of vibration exercise on the plasma antioxidant biomarkers and oxidative stress have rarely been studied and data on the safety and efficacy of vibration on plasma antioxidant biomarkers are lacking, in this report the effects of high-frequency loading by means of WBV on the antioxidant parameters in adult male Wistar rat model will be presented.

Materials and Methods

Male Wistar rats weighing 140–180 g, originating from different litter were obtained from Animal House of Physiology Group, Baqiyattallah University of Medical Sciences. Eight rats in each group (Control vs. Vibration) were randomly kept in plastic cages (four rats per cage) in a controlled environment with a 12-hour light/dark cycle and a constant temperature (22 ºC) and humidity (65–70%), with free access to food and water. Animals were weighed and provided clean cages weekly. Food intake was determined three times per week. Vibration training started after a week.

For vibration training, animals in the vibration group were placed in a compartment attached to a vibration platform (China). Training was performed as described by Naghii et al. (22). Rats were allowed to move freely in the cage during vibration. The vibrations generated by motors underneath the platform are transmitted to the animal on the machine.

Briefly, the vibration intervention for this group consisted of one–three 5-min cycles of vertical sinusoidal whole body vibration for four sessions in the first week and increased gradually to 45 min until day 24 (with three sessions per week); followed by 60 min per set for the next 20 sessions until the end of the 8th week of the experiment. Each training session was performed between 8.30–10.00 a.m.

After each vibration cycle, the animals were given 1–2 min rest break between cycles. The vibration was performed at mode 1 which is automatically programmed in the machine with an amplitude of 1–10 mm and at a frequency of 10–50 Hz. The acceleration and frequency of the machine in mode 1 in each cycle increases in a gradual, stepwise manner through a series of up and down directions and then they decrease with the same trend within each specific time period. The control animals remained in their cages and were placed on the vibration platform, without vibration treatment.

Eight weeks after the training program, rats from both groups were weighed after 12 h fasting and anesthetized for collection of blood by cardiac puncture with a syringe and needle. Rats were restrained from food for 12 h but had access to drinking water. All animals were euthanized immediately after blood sampling. Plasma samples were separated immediately and stored at –20 ºC until analysis, on the next day.

The plasma Cu and Zn concentrations, vitamin C, uric acid, the activities of antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPX], catalase, xantine oxidase), total antioxidant capacity (TAC), and malondialdehyde (MDA) levels were measured by commercially available assay kits.

Plasma Cu and Zn concentrations were determined by atomic absorption spectrophotometry (Chemtech Analytical, CTA-2000 AAS, Kempston, UK).

Vitamin C, xantine oxidase and catalase were assayed with commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

SOD activity and TAC were determined with kits from RANDOX Laboratories Ltd., Antrim, United Kingdom.

GPX (Enzymatic Colorimetric) and MDA level (lipid peroxidation; TBARS, Chemical colorimetric,) was assayed with commercial kits (Cayman Chemical Company, MI, USA). MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm.

Plasma uric acid concentrations were measured by an enzymatic photometric test using a kit obtained from Pars Azmun, Tehran, Iran.

Statistical analysis

Data are expressed as mean ± SD and a statistical package for the Social Sciences ([SPSS], New York: McGraw-Hill) was used to perform all comparisons. Independent sample *t*-tests were used to evaluate the effects of training between two groups. A *P*-value of less than 0.05 was considered significant for the differences. No multiplicity was performed for the comparisons.

Results

The rats in both groups adjusted to the vibration tolerated it and became acclimatized well with no signs of stress after one week. Furthermore, no major differences were observed in the amount of food and water intake between the control and vibration group the animals consumed food and water normally (Table I) and stayed healthy throughout the study.

The vibrated rats weighed more than the control group at the end of the study. They weighed 14% more (weight gain: 135.0 ± 21.0 vs. 157.0 ± 36.0 g, $P < 0.048$).

A significant difference ($P < 0.05$) was only observed in plasma levels of xantine oxidase between the vibration and control group (Table II). No major significant differences was noticed in the plasma Cu and Zn concentrations, vitamin C, uric acid, the activities of antioxidant enzymes, total antioxidant capacity, and MDA productions.

Table I. Food and water consumption, body weight of control and groups treated with vibration

	Control ($n = 8$)	**Vibration ($n = 8$)**
Food intake (g/d)	17.1	17.8
Water (ml/d)	20–25	20–25
Body wt (gr): 1st day	145.0 ± 10.0	149.0 ± 6.0
4 wks	229.0 ± 27.0	242.0 ± 34.0
8 wks	280.0 ± 31.0	306.0 ± 41.0
Difference in body wt or wt gain (gr)	135.0 ± 21.0	157.0 ± 36.0*

Table II. Effects of vibration training on plasma antioxidant parameters in control and vibration groups

Group / Variable	Control	Vibration	*P* value
Cu (ug/dl)	150.0 ± 8.50	140.0 ± 16.0	N.S
Zn (ug/dl)	132.0 ± 15.0	124.0 ± 16.0	N.S
SOD (U/ml)	159.0 ± 6.0	162.0 ± 7.0	N.S
GPX (mU/ml)	185.0 ± 22.0	188.0 ± 47.0	N.S
Catalase (U/ml)	4.30 ± 0.80	4.30 ± 0.70	N.S
Xan. oxidase (U/L)	3.90 ± 0.35	4.30 ± 0.40	0.05
TAC (mmol/l)	1.00 ± 0.04	1.01 ± 0.17	N.S
MDA (uM)	4.50 ± 0.45	5.00 ± 1.40	N.S
Vit. C (ug/ml)	4.17 ± 0.80	4.12 ± 0.60	N.S
Uric acid (mg/dl)	3.50 ± 0.70	3.55 ± 0.90	N.S

N.S = non-significant

Discussion

Following our published data on hematological parameters (22), it seemed to be well-advised to study the status of oxidative stress associated with WBV in an attempt to determine the influence of such a treatment. In our study the rats were healthy and endured the vibration with no signs of distress. The WBV group weighed 14% more after 8 weeks (weight gain: 135.0 vs. 157.0 g), while both groups consumed similar amount of food (17.1 vs. 17.8 g/d). It is assumed that the observed body weight gain may be the result of higher muscle mass and bone mass and/or the result of higher blood flow leading to the alteration on the peripheral vascular and tissue perfusion. Maddalozzo et al. reported that mature female vibrated rats weighed 10% less, with less body fat and serum leptin concentrations (20). In a study, Roelants et al. reported a significant increase in fat-free mass and strength following with whole body vibration. This study compared the effects of 24 weeks of resistance training and whole body vibration (frequency: 35–40 Hz; amplitude: 2.5–5 mm) on body composition and knee extensor strength in untrained young women. Although there were no significant changes in body weight or percent body fat in either group, the whole-body vibration group significantly increased fat-free mass (by 2.2%). A significant increase in knee extensor strength was also reported in both groups (26).

Moreover, findings focused on the benefits and efficacy of WBV on skeletal tissues and mass, particularly in enhancing muscle strength, balance, and mobility; and on bone mass and tissue architecture. It is reported that twenty-four weeks of WBV slightly increased lean mass in previously untrained females (27) and after 12 weeks reduced body fat accumulation and serum leptin levels with no alteration in food consumption (20). Similar findings from human studies confirm the effectiveness of WBV in improving health status such as, improving pain and fatigue in women with fibromyalgia (1), reducing the risk of bone fracture more than walking (16), increase in the serum levels of testosterone and growth hormone (6, 7, 17), and representing no stressful stimulus for the neuroendocrine and neuromuscular systems (11, 17). The influence of vibration on the bone mechanical properties revealed that the energy to maximal load for tibia bone was non-significantly higher (*P* = 0.09), and the stiffness of the femur bone plus the maximal load or breaking strength (F max) of the femur and lumbar bone in the treated group was non-significantly higher than the control group (21).

It is now assumed that evaluation on the issue of the effect of WBV on body weight requires a long-term study in which body composition consisting of fat, muscle and bone mass or metabolic factors in normal, overweight and obese rat needs to be determined.

Zinc and copper as two trace elements associated with adequate amount of antioxidant enzymes along with SOD were within normal levels with no alteration in any of the groups.

Whole body vibration has been shown to increase xanthine oxidase level with no significant change in plasma uric acid product in the vibrating group. Recent reports have focused on xanthine oxidase (XO) as a source of oxidative stress and have revealed that reactive oxygen species (ROS) are generated, at least in part, during the activation of xanthine oxidase (XO) (10, 24).

The evaluation of oxidative stress induced by vibration was performed by determining the levels of malondialdehyde derived from lipid peroxidation by the TBA method.

More evidently, these results suggest that the rise in xanthine oxidase level as a result of WBV induces no oxidative stress and biochemical impairments caused by MDA production. The findings are in accordance with the report of Siddique et al. showing that the evaluation of oxidative stress induced by vibration was performed by determining the levels of malondialdehyde derived from lipid peroxidation by the TBA method and the result revealed no perturbation in the oxidative stress status. Malondialdehyde (MDA) is used for the estimation of damage by reactive oxygen species and is a major reactive aldehyde resulting from the peroxidation of biological membranes. The most common method used to assess MDA production is the thiobarbituric acid (TBARS) assay (32).

The antioxidant enzymes produced within our bodies are complex proteins that often incorporate minerals such as selenium, zinc and Cu in their intricate structures. These antioxidant enzymes serve as the body’s most potent defense against free radicals and ensuing inflammatory reactions. They include glutathione peroxidase, catalase, and perhaps the most important internally generated antioxidant of all: superoxide dismutase (SOD). Primary antioxidants such as superoxide dismutase are our first and most important line of defense against highly reactive, potentially destructive oxygen-derived free radicals.

Exercise has been noted in some, but not all studies, to elicit an oxidative stress, depending on differences in exercise intensity across protocols, as well as differences in training status of participants. It appears that different types of exercise have different impact on the antioxidant system.

Conventional exercises such as endurance, ultra endurance exercise, downhill treadmill (muscle-damaging exercise) or a graded exercise treadmill test can increase oxygen utilization and greatly increase the generation of free radicals and enhance damage to muscles and other tissues (14, 18, 23, 33). However, no change in the analysis of oxidative stress in mice which were undergone a single session of aerobic exercise was reported (19). Acute exercise may play a beneficial role because of its ability to increase antioxidant defense mechanisms through a redox sensitive pathway (4).

In trained men strenuous bouts of exercise do not result in a significant increase in blood oxidative stress during the one hour post-exercise period (12).

In conclusion, while acute exercise triggers oxidative stress, chronic exercise has protective role against oxidative stress (3).

Exercise is any type of physical exertion we perform in an effort to improve our health, shape our bodies and boost performance. Obviously that covers a broad range of activities and, luckily, there are plenty to go around whether one wants to lose weight, get healthy or train for a sport.

Recently, whole body vibration has been proposed as a potential alternative, or adjuvant, to exercise (20). Vibration can be broken down into two basic components: magnitude and frequency. Magnitude encompasses displacement, velocity, acceleration and jerk. Currently, many companies advertise to use of WBV as an effective means by which muscle strength and bone mass (in addition to other physiological benefits) can be obtained. Both acute and chronic alterations in peripheral vasculature occur with WBV and other reports indicate that hormonal fluctuations resulting from WBV vary considerably. A comprehensive review on the potential effects of WBV on several physiological systems is presented by Prisby et al. (25). In summary, they provide some evidence of the effectiveness of WBV which may be related to tissue perfusion, fluctuations in systemic hormones, and/or occur via direct mechanical stimulation. However, there is still more data required to support these claims. One major problem is related to the applied vibratory protocols and experimental designs to make definitive conclusions. Therefore, understanding the physiological responses of the endocrine system may be imperative when deciphering the mechanisms involved for example in enhanced bone remodeling. Hormonal status subsequent to WBV has been measured and reported in our two groups, previously (21). Alterations in estradiol levels [E2 (pmol/l)] were found to be significant after vibration (30.65 ± 4.77 vs. 36.04 ± 8.07; $P = 0.031$).

WBV is implemented through the use of a vibrating platform on which exercises can be performed. The vibrations generated by the engines underneath the platform are transmitted to the person standing, sitting or lying on the machine. The amplitude and frequency of these vibrations can be set on the device.

It has been shown to increase muscular strength, explosive power and anabolic hormone levels when performed for as short a time as 4 minutes, three times a week (6). It requires relatively little exertion compared with traditional forms of exercise; yet studies comparing this training method to traditional strength training have found similar gains in strength and, in some cases, more gains in explosive power (27). Since whole body vibration training is of low impact, it may be a particularly good choice for older or obese clients who have trouble doing traditional weight-bearing exercise.

Conclusion

Several papers have been published on the effects of WBV, and the number of research studies conducted every year has been increasing. Effects described in the studies include: muscle strength and toning, cellulite reduction, improved bone density, heightened secretion of hormones associated with exercise, and depressed response of hormones associated with stress. Moreover, oxidative stress and antioxidant defense markers measured in this study were within normal levels, indicating that the potential effects of physiological responses to WBV on the antioxidant system are without deteriorations concerning plasma parameters. Even the rise in xanthine oxidase level was accomplished with no oxidative stress and biochemical impairment as shown in the MDA production. These findings may further promote eliciting scientific inquiries into this potentially therapeutic aid through further investigations on determining the optimal frequency, duration, amplitude, and appropriate protocols.

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